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TO: See Distribution List

FROM: Martha Lamont, Director
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SUBJECT: Microbiological Data Program Plan, January through June 2005

This Program Plan serves as the current Statement of Work for the period January 1, 2005 through June 30, 2005 for each State participating in the Microbiological Data Program (MDP). This document also stipulates work assignments for the Federal facility participating in MDP.

I. ADMINISTRATIVE UPDATES

Program participants are reminded to keep MDP management informed of any critical equipment purchases, staffing issues, or expected increases in rent or sample turn-around-time (e.g., due to laboratory or office renovation/relocation). This information is required under the terms of the MDP Cooperative Agreements (Section II, Responsibilities) between USDA and participating States.

The 2003 MDP Data Summary has been drafted and is under departmental review.

A. Personnel

Janet Doyle has been hired as an Interdisciplinary Scientist working on both MDP and the Pesticide Data Program.

B. Financial/Cooperative Agreements

A Continuing Resolution is in place until November 20, 2004. MDP anticipates that MDP FY05 funding may be established at $5.57 million, the same level as FY 2004. MPO is trying to avoid partial agreements; however, partials will be issued for States requiring funds if the budget is not passed by January 1, 2005. Final agreements will be issued when the budget is passed.

C. MDP Program Meetings

The next MDP Federal/State meeting will be held in June 2005, in Fairfax, VA.

D. Electronic Transfer of Data

RDE System Architecture: The Web-based RDE system is a centralized system, where all RDE database files and support software reside in Washington, D.C. and laboratory users require only an Internet web browser on the front-end for access. A stand-alone SIF data entry system for laptop/desktop computers and for palmtops (Pocket PCs) was developed to allow the capture of SIF data electronically by sample

collectors. The SIF data entry system can also be used by laboratories to perform off-line data entry of paper SIF information that can then be imported into the central RDE system. Laboratories that import complete data sets into the Web-based RDE system can request that HQ-MPO forward all incoming e-SIFs to the lab for import into the lab internal database or LIMS.

RDE Version Upgrades: A version upgrade for the Web-based RDE system is planned for March 2005. A version upgrade for the RDE e-SIF system for laptops/palmtops is planned for July 2005. A proposed stand-alone RDE system for off-line data entry at laboratories is presently under development and is expected to be ready by January 2005. The off-line RDE system will generate an output database holding completed sets/groups that can be directly imported into the Web-based RDE system. MPO maintains a Change Request Database to capture all problems identified and suggestions made regarding the RDE system.

RDE Web Addresses: RDE users in the laboratories should be using the SSL (Secure Socket Layer) site address to access the Web-based RDE system on the AMS production Web server. The only difference is the addition of the letter "s" following "http". This SSL technology is used to encrypt all data passed between the user's computer and the central web server. If the secure site is not available, the AMS developmental Web server can be used.

II. PROGRAM SAMPLING AND TESTING UPDATES

A. Sampling:

Shipping Charts are distributed quarterly to Sampling Managers by MPO.

Cantaloupe, tomatoes, green onions, parsley/cilantro and lettuce (leaf or romaine) will continue. Samples collected in Maryland will be sent to the Ohio laboratory (OH4) and those collected in Texas will be shipped to the AMS National Science Laboratory [NSL (US4)]. All other samples will be analyzed by the laboratory for that collection State.

B. Testing

Cantaloupe, tomatoes, green onions, parsley/cilantro and lettuce (leaf or romaine) will continue. Samples collected in Maryland will be tested by the Ohio laboratory (OH4) and those collected in Texas will be tested by NSL (US4). All other samples will be analyzed by the laboratory for that collection State.

Target Microorganisms

MDP laboratories will continue quantitative testing of all samples for *E. coli*. The method will be changed from ColiComplete to Fluorocult effective January 1, 2005. Method procedures are detailed in SOP MDP-MTH-01, *Escherichia coli* MPN Method.

MDP laboratories will continue to test all samples for *Salmonella* (presence or absence) by BAX®. Method procedures are detailed in SOP MDP-MTH-04, Detection of *Salmonella* in Fresh Produce using PCR-Based BAX® System

MDP laboratories will continue to test all samples for *E. coli* O157:H7 (presence or absence) by BAX®. Method procedures are detailed in SOP MDP-MTH-05, Detection of *Escherichia coli* O157:H7 in Fresh Produce using PCR-Based BAX® System. Presumptive positives are subjected to immunomagnetic separation (IMS) procedures and confirmed culturally, as described in SOP MDP-MTH-06, *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Presumptive Confirmation.

C. Quality Assurance

Proficiency Testing Program

ATCC will provide proficiency test sets for *Salmonella* in December 2004. Lyophilized cultures will be sent to all program laboratories and cultures will be reconstituted and diluted per MPO instructions by the onsite Quality Assurance Unit (QAU). Samples will then be inoculated by the QAU and issued to the technical group for analysis.

SOPs

SOPs are posted to the MDP website when distributed to program participants. http://www.ams.usda.gov/science/MPO/SOPs.htm.

The following SOPs were distributed August 1, 2004:

- MDP-LABOP-02 Sample Receipt and Elution Procedure (Revision 04,), Attachment, Fabrication of the California Cantaloupe Shaker Adapter
- MDP-LABOP-08 Procedure for Testing and Maintenance of Control Strains (Revision 01)

The following SOP was distributed October 1, 2004

- MDP-QA-03 Quality Assurance (QA) Controls (Revision 1); Attachment 01 Current and Historical QA Control Strain Information; and Attachment 02 QC Control Failure Reporting Form

The following SOP was distributed November 1, 2004:

- MDP-SHIP-03 Procedures for Packaging, Shipping, and Archiving Microbiological Cultures (Revision 01); Attachment 01, Shipment Destinations, Contacts, and Schedules; Attachment 02 MDP Participating Laboratories Addresses and Contact Staff for Shipment of Cultures; Attachment 03 Flowcharts of Shipments; and Attachment 04 MDP Shipping Form.

The following SOPs will be distributed December 1, 2004:

- MDP-DATA-01 Record Keeping and Results Reporting (Revision 02) and Attachment 01 Preliminary and Final Results Reporting Form
- MDP-DATA-02 Data Storage and Archival (Revision 01); Attachment 01 MDP Designated Federal Records Centers; Attachment 02 Standard Form (SF)-135 (template plus example); and Attachment 03 Instructions for Assembly and Packaging of Record Boxes

The following SOPs will be distributed January1, 2005:

- MDP-QA-03 Quality Assurance Controls (Revision 02); Attachment 01 Current and Historical QA Control Strain Information; and Attachment 02 QC Control Failure Reporting Form
- MDP-MTH-04 Detection of *Salmonella* in Fresh Produce using PCR-Based BAX® System Revision 01)
- MDP-MTH-05 Detection of *Escherichia coli* O157:H7 in Fresh Produce using PCR-Based BAX® System (Revision 01)
- MDP-MTH-06 *Escherichia coli* O157 Immunomagnetic Separation (IMS) Method and Presumptive Confirmation (Revision 01)

D. Archiving and Additional Testing

Archival of Isolates

The NSL, Gastonia, NC has been established as a centralized location for archival of isolates as well as a distribution center for isolates from MDP testing laboratories to the reference laboratories.

Additional Testing by Reference Laboratories

Samples that tested presumptive positive for *E. coli* by the MUG-based MPN method are shipped directly to the Florida laboratory for multiplex PCR (mPCR). These samples are further screened for shiga toxin producing *E. coli* (STECs), enterohemorrhagic *E. coli* (EHECs), and enterotoxigenic *E. coli* (ETECs). Isolates of pathogenic *E. coli* are archived in Microbank™ vials and shipped to NSL.

E. coli isolates are shipped by NSL to Pennsylvania State University for serotyping and to the FDA/CVM laboratory in Laurel, MD for antimicrobial resistance testing and inclusion in the National Antimicrobial Resistance Monitoring System (NARMS) and pulsed field gel electrophoresis (PFGE) analysis for inclusion in PulseNet.

Salmonella and *E. coli* O157 isolates are frozen in two Microbank™ vials which are shipped to NSL. Isolates are then shipped to FDA/CVM for antimicrobial resistance testing, PFGE, and inclusion in PulseNet, and serotyping.

Data Transfer

AMS will transfer data to CDC and FDA on a semi-annual basis.

E. Training

MPO implemented a week-long training course for MDP laboratory personnel, September 20-24, 2004. A full report on the training was distributed on October 26, 2004. Due to the positive feedback received for this class, future courses for MDP laboratory staff will be conducted on an "as needed" basis.

Based on the analysis of the responses to the course evaluation the following recommendations for improvement were presented:

- Limit the size of each class to no more than ten participants.
- Conduct separate courses for experienced and novice participants.
- Tailor the curriculum around the experience level of the participants.

- Conduct three-day workshops on molecular methods.
- Provide a brief introduction to each "hands-on" laboratory exercise before commencement, followed by discussion after the completion of the exercise.
- Conduct a more thorough assessment of potential hotel accommodations.

F. Future Program Directions

Universal Pre-enrichment Broth (UPB): Current MDP methods use different pre-enrichment broths for growing target bacteria. This requires additional personnel, time, and materials, thereby increasing analytical costs. UPB has been shown to be an ideal broth for enriching *Salmonella*, *Listeria*, and *E. coli* O157:H7 and for reviving sub-lethally damaged cells. Using a single pre-enrichment broth such as UPB for culturing different bacteria will streamline this labor-intensive step and subsequent analyses in the screening process. MPO is collaborating with the Wisconsin laboratory on UPB method development work.

Sprouts are being investigated as a replacement commodity for cilantro/parsley beginning July 2005. Alfalfa/clover sprouts will be tested for all required organisms. MPO is collaborating with the Ohio laboratory on method development that includes stomaching of the sprouts and a DNA clean-up step.

Multiplex PCR is currently performed by the Florida laboratory on all program-wide samples testing presumptive positive for *E. coli*. This technology will be transferred to all program laboratories in the future.

Shigella: MDP will continue to work on the implementation of *Shigella* testing for all commodities, including alfalfa sprouts. This method development will include enrichment, DNA clean-up, and cultural identification steps. Initial method performance studies were investigated by the Minnesota laboratory.

Prior to program-wide introduction, all methods will be tried out by the participating laboratories.